A genetic association study of the IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam study)

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CONCISE REPORTS

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Abstract

***Objective*—A genetic association study was performed to investigate whether radiographical osteoarthritis (ROA) was associated with specific genotypes of the insulin-like growth factor I (IGF-1) gene.**

***Methods*—Subjects aged 55–65 years were selected from a population-based study of which ROA at the knee, hip, spine, and hand was assessed. Genotypes were determined of a polymorphism in the promoter region of the IGF-1 gene.**

***Results*—The IGF-1 locus was significantly associated with the presence of ROA (overall adjusted OR for heterozygous subjects = 1.9, 95% CI 1.2, 3.1 and for homozygous subjects 3.6, 95% CI 0.8, 16.2).**

***Conclusion*—These results suggest that variation at the IGF-1 locus is associated with ROA development and may play a part in ROA pathogenesis. To confirm these findings replication in another population-based sample is needed.**

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Osteoarthritis (OA) is a disease characterised by the degradation of articular cartilage and formation of new bone (osteophytes and sclerosis). Several reports suggest that genetic influences contribute considerably to the development of OA.[1 2] The relevance of the genetic component, however, varies among subgroups of patients and as yet it is not clear which genes are involved.[3]

Insulin-like growth factor 1 (IGF-1) stimulates chondrocytes to synthesise extracellular matrix (ECM) components in cartilage.[4 5] Its action is mediated through the type 1 IGF receptor. The function of IGF-1 and its receptor in cartilage formation both during developmental stages and remodelling of adult cartilage may be relevant to the aetiology of OA. IGF-1 may also influence OA by osteophyte formation.[6] Although osteophyte growth during OA progression and bone mineral density (BMD) is positively correlated to high serum IGF-1 concentrations,[6 7] no consistent relation between serum IGF-1 concentrations and OA has been found.[8 9] Furthermore, serum IGF-1 is inversely correlated to age[10] and body mass index (BMI).[11] As individual IGF-1 concentrations are liable to temporal variations, assessing the role of IGF-1 in OA by serum concentrations is complex. We have examined the relation between the IGF-1 gene and the presence of radiographical OA (ROA) in subjects aged 55–65 years.

Methods

SUBJECTS

Subjects were derived from a prospective population-based cohort study of determinants and prognosis of chronic diseases in the elderly, the Rotterdam study.[12] Weight bearing anterior-posterior radiographs of the hips and knees, anterior-posterior radiographs of the hands and wrists, and lateral radiographs of the spine (Th4-S1) were obtained from a random cohort of 1040 unrelated people aged 55–65 years (425 men and 615 women). ROA was assessed by two independent readers and by means of the grading system proposed by Kellgren.[13] By applying these criteria a hip joint with Kellgren score 2 requires the presence of both definite joint space narrowing and definite osteophytes. A knee or hand joint with Kellgren score 2 requires the presence of definite osteophytes and possible narrowing of joint space. A spine joint with Kellgren score 2 denotes definite lateral osteophytes. For both knees and hips radiographs had previously been scored.[14] ROA of the hand was assessed in each inter- and metacarpalphalangeal joint individually, and the first carpometacarpal and trapezoscaphoideal joint. ROA of the wrist was assessed at the radiocarpal and distal radioulnar joints. For the spine three levels, from Th4 to S1, were scored with regard to osteophytes and disc space narrowing, for example, thoracic, lumbar and lumbosacral. In the analysis definite ROA was defined as Kellgren score 2 or over in the left and/or right corresponding joint. Hand ROA was defined as Kellgren score 2 or over in at least one of the 36 joints that were scored. For this purpose the joints of the wrist were included in the category hand ROA. ROA of the spine was defined as Kellgren score 2 or over in at least one out of three levels scored. The presence or absence of

Table 1 Population description ERGO cohort of 55–65 years and allele frequencies of the dinucleotide repeat polymorphism in the promoter region of the IGF-I gene

		Overall	*ROA+*	*ROA−*		
Subjects (n)		786	651	135		
Characteristics of subjects						
Men (% of total)		317 (40)	254 (39)	63 (47)		
Age (y) (SD)			60.5 (2.7)*	59.5 (2.7)		
BMI (kg/m^2) (SD)			26.6 (3.7)*	25.1 (3.1)		
BMD (g/cm^2) (SD)			0.87 (0.13)	0.86 (0.12)		
Allele frequencies (no of alleles)						
Alleles†	Weber‡ (88)	Overall (1572)	ROA+ (1302)	ROA− (270)	Λ§	p
A2	0.18	0.07	0.07	0.07	4.9	0.02
A3	0.16	0.19	0.20	0.13		
A4	0.60	0.66	0.65	0.72		
A5	0.06	0.05	0.05	0.05		
other	0.00	0.04	0.04	0.04		

*Significantly different from the control group with p<0.01. †IGF-I alleles with nomenclature as in Weber and May.[15] ‡Allele frequencies as in Weber and May.[15] §Likelihood-ratio test statistic = $-2\ln(L(H_0)/L(H_1))$.

Table 2 Odds ratios of subjects with IGF-I genotypes containing allele 3 compared with all other genotypes

	No of A3 genotype (frequency)				
Subjects	*−/−*	*−/+*	*OR (95% CI)**	*+/+*	*OR (95% CI)**
ROA−	103 (0.76)	30 (0.22)	1	2 (0.01)	1
ROA+	417 (0.64)	211 (0.32)	1.9 (1.2, 3.1)	23 (0.04)	3.6 (0.8, 16.2)

*Odds ratio adjusted for age, body mass index, bone mineral density.

Table 3 Odds ratios of subjects with IGF-I genotype containing allele 3 (homo and heterozygote) compared with all other genotypes

Subjects	*Number*	*Number of other genotypes (frequency)*	*Number of A3 genotypes (frequency)*	*Crude OR (95% CI)*	*Adjusted OR (95% CI)**
Total	786	520 (0.66)	266 (0.34)		
ROA−	135	103 (0.76)	32 (0.24)	1	1
ROA+	651	417 (0.64)	234 (0.36)	1.8 (1.2, 2.8)	2.0 (1.3, 3.1)
Knee ROA	142	96 (0.68)	46 (0.32)	1.5 (0.9, 2.6)	1.8 (1.0, 3.4)
Hip ROA	71	42 (0.59)	29 (0.41)	2.3 (1.2, 4.2)	2.8 (1.4, 5.7)
Hand ROA	444	284 (0.64)	160 (0.36)	1.8 (1.2, 2.9)	2.2 (1.4, 3.5)
Spine ROA	479	308 (0.64)	171 (0.36)	1.8 (1.2, 2.9)	2.0 (1.2, 3.2)

*Odds ratio adjusted for age, body mass index, bone mineral density.

osteophytes of the knee were scored separately. Information on age (in years), BMI (measured as weight in kg divided by height^2 in metres), and BMD (measured as gram mineral divided by area in cm^2) of the neck of the femur were used in the study. In view of the hypothesis that the genetic contribution to ROA differs in men and women and may depend on the joint site, stratified analysis was performed with respect to sex and joint site.

GENOTYPING AND STATISTICAL ANALYSIS

Genotypes of the dinucleotide repeat polymorphism of the IGF-1 gene were determined as previously described[15] of 786 people for whom cells were available. Patient characteristics (sex, age, BMI, and BMD) were compared between subjects with and without ROA by using *t* tests for independent samples. Allele frequencies were assessed by counting alleles and calculating sample proportions. The χ^2 test for Hardy Weinberg equilibrium (HWE) was calculated using the HWE-program (LINKUTIL package).[16] Alleles with an allele frequency < 0.05 were pooled. A likelihood ratio test was used to test for association of IGF-1 alleles with the occurrence of ROA.[17] This method is specifically suitable to perform association studies with polymorphic markers with multiple alleles. To measure the strength of association between ROA and IGF-1 genotypes, a logistic regression model was used to estimate the odds ratio (OR). ORs were adjusted for risk factors of OA—that is, sex, age, BMI and BMD. ORs are presented with 95% confidence intervals (95% CI). The statistical package SPSS was used and p values < 0.05 (two sided) were considered significant.

Results

Table 1 shows the number of genotyped people with ROA (ROA+) and without ROA (ROA−) in any of the joint sites investigated and the mean age, BMI, and BMD. The mean age and BMI differed significantly between subjects with and without ROA (p < 0.001). Only 17% were free of ROA in every joint investigated in this relatively young age group.

In total nine different alleles were identified (A1–A9) with allele frequencies ranging from 0.002–0.66. IGF-1 allele frequencies of the four most frequent alleles are shown in table 1 for the random cohort, for ROA+ and for ROA− subjects. Except for the relatively rare allele A2, the IGF-1 allele frequencies observed in the cohort, and the subjects with and without ROA were similar as described by Weber and May[15] (see table 1). The low frequency alleles A1 (frequency 0.02), A6 (frequency 0.02), A7 (frequency 0.003), and A9 (frequency 0.002) (not shown in table 1) were not previously described in a population-based study.[15] The distribution of genotype frequencies was not significantly different from that expected for a population in HWE neither overall (p = 0.76) nor for the ROA+ (p = 0.78) or the ROA− group (p = 0.47).

Table 1 shows association of the IGF-1 polymorphism in ROA+ as compared with ROA− subjects (p = 0.02). This association was caused by differences in allele frequency A3 and A4 between ROA+ and ROA− subjects. The strength of the association of the IGF-1 locus with ROA was estimated using a logistic regression model (table 2). The overall adjusted OR (age, sex, BMI, and BMD) for ROA+ subjects heterozygous for IGF-1 allele A3 was 1.9, 95% CI 1.2, 3.1. For subjects homozygous for IGF-1 allele A3 (A3/A3) the overall adjusted OR (age, sex, BMI, and BMD) was 3.6, 95% CI 0.8, 16.2. A protective effect was observed for the IGF-1 allele A4 with an overall adjusted OR (age, sex, BMI, and BMD) for heterozygous ROA+ subjects of 0.7, 95% CI 0.4, 1.5 and an overall adjusted OR of 0.5, 95% CI 0.3, 1.0 for homozygous A4/A4 subjects. Interaction in these analyses with sex, age, BMI, and BMD was not observed.

As IGF-1 allele A3 shows a significant OR and is rarer than allele A4 it is, from a population genetic point, the most likely allele associated to ROA. We, therefore, have chosen to further investigate the effect of allele A3. To retain sufficient numbers (power) the homo and heterozygous genotypes with allele A3 were added and used to perform stratified analysis by sex and by separate joint sites in ROA+ subjects. The overall adjusted OR calculated for homo and heterozygous carriers of the A3 allele together using the remaining

genotypes as reference was 2.0, 95% CI 1.3, 3.1. This association with IGF-1 A3 genotypes was found in both men (adjusted OR = 2.4, 95% CI 1.2–4.8) and women (adjusted OR = 1.8, 95% CI 1.0–3.3) being strongest in men. When subjects were selected on joint site specific ROA, a significant effect of the IGF-1 genotype with allele 3 was observed for each individual joint (table 3). The strongest effect as measured by the OR was observed in subjects with hip ROA with an adjusted OR of 2.8, 95% CI 1.4, 5.7. When subjects were stratified by number of joint groups affected (knee, hip, hand, and spine) the strength of the association measured by the OR of A3 genotypes was not higher for subjcets with ROA in ⩾ 3 joint sites (generalised ROA).

Discussion

We investigated whether a polymorphic marker of the IGF-1 gene is associated with the presence of ROA at knee, hand, hip, and spine. In a population-based cohort of 786 subjects, the frequency of the subjects heterozygous for the IGF-1 allele A3 was found to be approximately two times increased in ROA+ subjects compared with ROA− subjects (adjusted OR 1.9, 95% CI 1.2, 3.1). A 3.5 times increased frequency of the A3/A3 homozygous IGF-1 genotype was observed among subjects with ROA+ (adjusted OR 3.6, 95% CI 0.8, 16.2). The latter association was not significant, probably because of the small number of subjects homozygous for IGF-1 allele 3 (for ROA− n= 3, for ROA+ n=23). The associations observed were not explained by age, sex, BMD, or BMI.[7 10 11] The deviation of IGF-1 allele A4 may be a consequence of compensating allele frequencies. Moreover, no association was found between IGF-1 alleles and BMD or BMI nor did we observe an association of IGF-1 locus with the presence of osteophytes in the knee as has been suggested for serum IGF-1 concentrations[6] (results not shown). As association studies are subject to false positive results the observed associations requires cautious interpretation and replication in a second population-based study.

In view of the function of IGF-1 in articular cartilage metabolism and the intragenic location of the dinucleotide repeat polymorphism, the association found may be because of the role of the IGF-1 gene in the onset of ROA. We, however, cannot exclude the possibility that a gene closely linked to the IGF-1 locus, influences the association. Two other genes on chromosome 12 that may play a part in the onset of OA are the procollagen type II (COL2A1)[18] and the vitamin D receptor (VDR) genes.[19] The location of these genes, however, is on 12q12–14, which is at least 80 cM of the IGF-1 gene. As in unrelated subjects linkage disequilibrium extends only over short genetic distances (1–2 cM), it is not likely that mutations in these genes cause the currently described associations.

In addition to IGF-1, genotypes of a polymorphism in the gene encoding the receptor of the IGF-1 gene[20] were studied but did not show any association in subjects with ROA in the knee and/or the hip as compared with subjects without ROA (results not shown).

Although the IGF-1 polymorphism is located in the promoter region, and may affect the expression of the gene, its relation to IGF-1 concentrations is not yet known. The association observed may be explained by differences in carriers versus non-carriers in the response of the chondrocyte, via IGF-1, to cartilage damage and degradation during the OA process.[4 5] The observation that IGF-1 allele 3 is associated with ROA at any joint site (knee, hip, hand, and spine) and does not increase specifically for subjects with generalised ROA may indicate a mild genetic predisposing effect, which phenotypic outcome may depend on other factors, for example, mechanical stress. Carriers of the IGF-1 allele 3 may thus be predisposed to ROA at any possible joint.

The observed association was strongest in subjects with hip ROA, a site for which the role of genetic factors was not previously assesed. It has, however, been reported that ROA of the hip has a specific sex and geographically prevalence pattern, which may suggest the involvement of systemic factors to the onset of hip ROA. Furthermore, hip ROA is often considered to arise because of anatomical abnormalities.[21] As IGF-1 is expressed during developmental stages and plays an important part in cartilage formation,[22] the effect of the IGF-1 locus may be exerted via this way.

In our population-based study we were able to study specifically the pathophysiology of ROA of the hip, knee, hand, and spine. Our selection criteria for ROA negative subjects were strict in that we included only subjects without ROA at any of the four joints studied. Because of the high prevalence of hand and spine ROA only 135 (17%) ROA negative subjects fulfilled these criteria. This may have contributed to the detection of association because the observed association was mainly a result of the reduced frequency of allele A3 in subjects without ROA, suggesting that the absence of this allele in particular in the genotype lowers the risk for ROA. It is also possible that another IGF-1 allele protects subjects without ROA. The frequency of IGF-1 allele 4 in this respect, is higher among subjects without ROA and may possibly protect. The relative high allele frequency of this allele, however, may have decreased power to significantly prove such a protective effect. The independent effect of allele A3 and A4 could not be tested by exclusion of subjects with either IGF-1 because the remaining number of subjects was not sufficient.

Our study, shows an association of IGF-1 genotype with the prevalence of ROA in knee, hip, hand, or spine irrespective of BMD, BMI, and age. These findings suggest that IGF-1 plays a part in OA pathogenesis.

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